AxyPrep Mag™ PCR Clean-up Protocol

Introduction

The AxyPrep Mag PCR Clean-up kit utilizes a unique paramagnetic bead technology for quick high-throughput purification of PCR amplicons. AxyPrep Mag PCR Clean-up utilizes an optimized buffer to selectively bind PCR amplicons 60bp and larger to paramagnetic beads. The protocol mainly consists of binding, washing and elution steps. Primers, nucleotides, salts and enzymes in reaction mixture are removed during the binding and washing steps. The purified PCR product is essentially free of contaminants. Furthermore, primer dimers can be removed by using reduced concentration of AxyPrep Mag PCR Clean-up.

AxyPrep Mag PCR Clean-up can be used in the following applications

- PCR
- Sequencing (Sanger and Next Generation)
- Fragment Analysis
- Primer walking
- Genotyping and SNP detection
- Restriction enzyme clean-up
- Cloning
- Manual and automation friendly

Product Highlights

• Flexible Clean-up: High recovery of amplicons of 100 bp and higher
• Efficient removal of unincorporated dNTPs, primers, primer-dimers, salts and other contaminants
• Manual and automation friendly protocol
• Processing time: 15 mintues/96 samples
• No centrifugation or filtration required
• Scalable: Tube, 96 and 384 well plate

Process Overview

1. Add 18μL from AxyPrep Mag PCR clean-up per 10μL of PCR product for DNA binding to Magnetic beads.
2. Separate beads and DNA from unbound contaminants.
3. Wash beads twice with 70% ethanol to remove salt and contaminants
4. Elute purified PCR products from Magnetic beads to a new plate

Kit Specifications

The AxyPrep Mag PCR Clean-up kit can be performed in a tube, 96 well- and 384 well-formats. The following table illustrates the number of PCR reactions a AxyPrep Mag PCR Clean-up kit can purify depending on the PCR reaction volume.

AxyPrep Mag PCR Clean-up Kits

AxyPrep Mag PCR Clean-up Products	P/N
AxyPrep Mag PCR Clean-up - Small 5 mL	Mag-PCR-CL-5
AxyPrep Mag PCR Clean-up - Medium 50 mL	Mag-PCR-CL-50
AxyPrep Mag PCR Clean-up - Large 250 mL	Mag-PCR-CL-250

PCR Reaction Volume **(96 well, µL)**	MAG-PCR-CL-5 **(# reactions)**	MAG-PCR-CL-50 **(# reactions)**	MAG-PCR-CL-250 **(# reactions)**
10	305	3055	15275
20	152	1527	7635
50	61	611	3055

Materials Supplied in the Kit

- **AxyPrep Mag PCR Clean-up** paramagnetic bead Solution
- **Store at 4°C upon arrival (not freeze), for up to 12 months**
- **Mix the reagent well at room temperature** to completely resuspend beads prior to research use. It should show homogenous in visual appearance.

Materials supplied by the User:

Consumables & Hardware:

Name	Recommended Model	Recommended Vendor and P/N
96-well PCR reaction plate	96-well round/ flat bottom microtiter plate. Plate selection depends on the PCR reaction volume	Corning, Inc., www.corning.com # 3797, 96 well round bottom # 3591, 96 well flat bottom # 3957, 0.5mL v bottom 96 # 3365, 360µL round 96 # 3364, 360µL flat 96 # 3371, 96 clear pro
	96-well cycling plate	Axygen, PCR-96-FS-C, PCR-96M2-HS-C, www.axygen.com
384-well PCR reaction plate	384 well cycling plate	Axygen, PCR-384M2-C, www.axygen.com
PCR Plate Seals	Easy Peel Heat Sealing Foil	Axygen, MF-111, www.axygen.com
Liquid handling robotics	Compatible with open platform robotics	Contact Axygen Biosciences Technical support for compatible AxyPrep Mag methods and accessories to your automation
Multichannel hand pipette	AxyPet	Single, 8 and 12 Multichannel

Handheld Magnetic Separation Devices Selection Guide:

The handheld Magnetic devices have been optimized for different AxyPrep Mag protocols. These Magnets address different volumes for tubes and plate types.

Protocol	Manufacturer	Part number	Plate description	Plate Material	Part Number
AxyPrep Mag Kits	Axygen	SCT-050-SS-C	0.5 ml Self Standing Screw cap tube	Polypropylene	IMAG-12T
	Axygen	SCT-150-SS-C	1.5 ml Self Standing Screw cap tube	Polypropylene	
	Axygen	SCT-200-SS-C	2.0 ml Self Standing Screw cap tube	Polypropylene	

Protocol	Manufacturer	Part number	Plate description	Plate Material	Part Number
AxyPrep Mag PCR Clean -up AxyPrep FragmentSelect-I AxyPrep FragmentSelect-R AxyPrep Mag DyeClean AxyPrep Mag DNA Normalizer AxyPrep Mag Blood gDNA AxyMag FFPE (DNA-RNA- miRNA) AxyPrep Mag Plasmid Kit AxyPrep Mag Tissue gDNA	Corning	3364	96 flat 360ul	Polypropylene	IMAG-96P
	Corning	3591	96 flat bottom	Polystyrene	
	Corning	3365	96 round 360ul	Polypropylene	
	Corning	3371	96 clear pro round	Polypropylene	
	Corning	3797	96 round bottom	Polystyrene	
	Corning	3957	96 v bottom 0.5mL	Polypropylene	
	Axygen	PCR-96-FS-C	96 PCR full skirt	Polypropylene	
	Axygen	PCR-96M2-HS-C	96 PCR half skirt		
	Corning	3959	96 round bottom 1ml		
	Corning	3961	96 round bottom 2ml		

Reagents

Reagents	Application
70% ethanol	Washing solvent
10 mM TRIS-HCl, pH=8.0	DNA elution
reagent grade water	
10 mM Tris-HCl pH 8.0, 1 mM EDTA	
Ethanol	Prepare ready to use AxyPrep Mag Clean-up

Procedure in 96 Well Format:

1. Determine whether or not a plate transfer is necessary.

If the PCR reaction volume multiplied by 2.8 exceeds the volume of the PCR plate, a transfer to a 300µL round bottom plate is required. To remove DNA dimer smaller than 100bp, 1.2X may be used. Alternatively, 2M NaCl may be used to dilute the beads proportionally and used at 1.8X.

2. Gently shake the AxyPrep Mag PCR Clean-up bottle to resuspend any Magnetic particles that may have settled. Add AxyPrep Mag PCR Clean-up according to the PCR reaction volume table below:

PCR Reaction Volume (µL)	**AxyPrep Mag PCR Clean-up** Volume at 1.8X (µL)
10	18
20	36
50	90

Note: The volume of **AxyPrep Mag PCR Clean-up** for a given reaction can be determined from the following equation: (Volume of **AxyPrep Mag PCR Clean-up** per reaction) = 1.8 x (PCR Reaction Volume)

3. Mix reagent and PCR reaction thoroughly by pipette mixing 5 times.

4. Incubate the mixed samples for 5 minutes at room temperature for maximum recovery.

This step allows the binding of PCR products 125bp and greater to the Magnetic beads. After mixing, the color of the mixture should appear homogenous.

5. Place the reaction plate onto a 96 well Magnet Plate for 3 minutes or wait until the solution is clear. Wait until the solution is clear before proceeding to the next washing step. Otherwise there may be beads loss.

6. Aspirate the cleared solution from the reaction plate and discard

This step must be performed while the reaction plate is placed on the 96 Magnet Plate. Avoid disturbing the settled Magnetic beads. If beads are drawn into tips, leave behind a few microliters of solution.

7. Dispense 200µL of 70% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate out the ethanol and discard. Repeat for a total of two washes.

It is important to perform these steps with the reaction plate on a 96 well Magnetic Plate. Do not disturb the settled Magnetic beads. Remove all of the ethanol from the bottom of the well to avoid ethanol carryover.

NOTE: A 5 min air dry at room temperature is recommended for the evaporation of the remaining traces of ethanol. Do not overdry the beads (the layer of settled beads appears cracked) as this will significantly decrease elution efficiency.

8. Take off the plate from the Magnetic plate, add 40µL of elution buffer (Reagent grade water, TRIS-HCl pH 8.0, or TE buffer) to each well of the reaction plate and pipette mix 5 times.

More than 40µL of elution buffer can be used, but using less than 40µL will require extra mixing to ensure the liquid comes into contact with the beads and may not be sufficient to elute the entire PCR product. Elution is quite rapid and it is not necessary for the beads to go back into solution for it to occur.

9. Place the reaction plate onto a 96 well magnetic plate for 1 minute to separate beads from the solution.

10. Transfer the eluate to a fresh plate for storage and analysis.

Protocol for the 384 Well Format:

1. Prepare ready to use AxyPrep Mag PCR Clean-up by adding appropriate amount of ethanol based on the following table:

AxyPrep Mag PCR Clean-up Products	ethanol (mL)
AxyPrep Mag PCR Clean-up -Small 5.5mL	0.75
AxyPrep Mag PCR Clean-up - Medium 55mL	7.5
AxyPrep Mag PCR Clean-up - Large 275mL	30

2. Shake the AxyPrep Mag PCR Clean-up bottle to resuspend any Magnetic particles that may have settled. Add AxyPrep Mag PCR Clean-up according to the following PCR reaction volume table:

PCR Reaction Volume (µL)	AxyPrep Mag PCR Clean-up Volume (µL)
5	9
7	12.6
10	18
14	25

Note: The volume of AXYPREP Mag PCR CLEAN-UP for a given reaction can be determined from the equation: (Volume of **AxyPrep Mag PCR Clean-up** per reaction) = 1.8 x (PCR Reaction Volume).

NOTE: Due to the constraint of the total volume of PCR reaction plus reagent, it is not possible to purify PCR reactions larger than 14µL within the well of 384 well plates (14µL reaction + 25µL **AxyPrep Mag PCR Clean-up** = 39µL).

3. Mix reagent and PCR reaction thoroughly.
Pipette mix 15 times. The color of the mixture should appear homogenous after mixing.

4. Place the reaction plate onto a 384 magnetic plate for 1 minute to separate the

beads from solution.

Wait until the solution is clear before proceeding to the next step.

5. Aspirate the cleared supernatant from the reaction plate and discard.

This step should be performed while the purification plate is situated on the 384 well Magnetic plate. Do not touch the Magnetic beads, which have formed a spot on the side of the well.

6. Dispense 30µL of 70% ethanol wash solution to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate the ethanol out and discard. Repeat for a total of two washes.

It is important to perform these steps with the reaction plate situated on a 384 well Magnetic plate. Do not disturb the separated Magnetic beads. Be sure to remove all of the ethanol from the bottom of the well as it is a known PCR inhibitor.

NOTE: A drying time of 5 min at Room Temperature is optional to ensure all traces of ethanol are removed but take care not to over dry the bead (beads appears cracked) as this will significantly decrease elution efficiency.

7. Off the Magnet plate, add 30µL of elution buffer (Reagent grade water, TRIS-HCl pH 8.0, or TE) to each well and pipette mix 10 times.

A 30µL elution volume will ensure the liquid level will be high enough to contact the Magnetic beads. A greater volume of elution buffer can be used, but using less than 15µL requires extra mixing (to ensure the liquid comes into contact with the beads) and may not fully elute the entire product. Elution is quite rapid and it is not necessary for the beads to go back into solution for it to occur.

When setting up downstream reactions, pipette the DNA from the plate while it is situated on a 384 well Magnetic plate. This will prevent bead carry over (however, beads will not inhibit thermal cycling reactions).

SUPPLEMENTAL PROTOCOL

Recovery of DNA smaller than 100bp

For best recovery of DNA smaller than 100bp, there are two options: ethanol or Isopropanol may be added to the **AxyPrep Mag PCR Clean-up** reagent or to the final bead-sample mix.

1. Add ethanol or Isopropanol to the sample-bead mix to a final concentration of 25%.

2. Add ethanol or Isopropanol to beads directly prior to starting the purification process. 70µL of Isopropanol should be mixed with 180µL of **AxyPrep Mag PCR Clean-up** bead solution. For every 10µL of PCR product, add 25µL of bead-Isopropanol mix. If using automation for PCR product cleanup, set the PCR product to 1.4 fold of the actual volume. For instance, if there is 10µL of PCR for clean-up; set the PCR sample volume to 14µL instead so that 1.4 folds of more bead-Isopropanol reagent will be added by the robot.

Removal of primer dimer or DNA smaller than 1kb

If longer primer sets are to be used and there is primer dimer, it is possible to remove primer dimer by using diluted **AxyPrep Mag PCR Clean-up** reagents.

1. Do a sequential titration to determine the best dilution for your PCR product. Add 2µL, 4µL, 8µL into 18µL of **AxyPrep Mag PCR Clean-up** reagents and test for primer dimer or DNA removal.

2. Use that dilution for subsequent PCR purification.

AXYPREP Mag PCR CLEAN-UP protocol for 96 Well Format

1 Sample volume x 2.8 > Well volume? _ Yes, go to step 2 _No, go to step 3

2 Transfer sample to a 300µL round bottom plate.

3 Shake the AxyPrep Mag PCR Clean-up bottle to fully resuspend Magnetic particles.

4 Add Sample Volume x 1.8 of AxyPrep Mag PCR Clean-up. Pipette mix 10 times.

5 Incubate at room temperature for 5 minutes.

6 Place the reaction plate onto a 96 well magnetic plate for 30 seconds to separate beads from solution.

7 Aspirate the supernatant from the reaction plate and discard.

8 Dispense 200µL of 70% ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard. Repeat for a total of two washes.

9 Add 40µL of elution buffer, pipette mix 10 times.

10 Incubate at room temperature for 2 minutes.

11 Place the reaction plate onto a magnetic plate for 1 minute to separate beads from solution.

12 Transfer purified product to a fresh plate

Typical reaction volume is 10–20 µL for the 96-well microplate and 5–10 µL for the 384-well microplate. Number of preps is based on 10µL reaction volume.

Please contact Axygen Biosciences for sales support at: axgsales@corning.com and for technical support at: axgsupport@corning.com

** The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.*